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Influence of dietary levels of lipid and vitamin E on growth and resistance of Nile tilapia to *Streptococcus iniae* challenge

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ABSTRACT

A study was conducted to determine the effect of dietary levels of lipid and vitamin E on growth performance, immune responses and resistance of Nile tilapia to *Streptococcus iniae* challenge. A basal purified diet (35% protein and 3.4 kcal DE/g) supplemented with 6, 10 and 14% of 1:1 mixture of corn oil and menhaden fish oil was each supplemented with 50, 100 and 200 mg vitamin E/kg. Each diet was fed to Nile tilapia in triplicate aquaria for 12 weeks. Weight gain, feed intake and survival were not affected by dietary levels of either lipid or vitamin E. Feed efficiency in fish fed 14% lipid diets was significantly lower than that fed 6% dietary lipid but these did not differ from that of the 10% dietary lipid diet. These variables were not affected by dietary vitamin E levels. Whole body lipid significantly increased in fish fed 14% lipid diets and 100 mg/kg vitamin E diets. Liver α-tocopherol levels were reflective of dietary levels of vitamin E. Increasing dietary levels of lipid to 14%, however, significantly decreased liver concentration of α-tocopherol. Hematological parameters and hepatosomatic indices were not affected by dietary treatments. Serum protein significantly increased in fish fed 14% lipid diets but was not affected by supplemental levels of vitamin E. Lysozyme activity was not affected by dietary lipid levels but significantly increased in fish fed 200 mg vitamin E diets. Alternative complement activity significantly decreased in fish fed 10 or 14% dietary lipids but increased when dietary vitamin E levels was increased to 100 or 200 mg. Dietary lipid and vitamin E levels had no effect on the resistance of Nile tilapia to *S. iniae* infection and on antibody titer against that bacterium.

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1. Introduction

Dietary lipids are an important source of highly digestible energy and are the only source of essential fatty acids required by fish for normal growth, development and maintaining health. Tilapia have been shown to require a dietary source of linoleic ($n-6$) series fatty acids (Kanazawa et al., 1980; Takeuchi et al., 1983; Santiago and Reyes, 1993; Yildirim-Aksoy et al., 2007). Linolenic ($n-3$) series of fatty acids may also be dietary essential for tilapia, but the required levels have not been determined (Lim and Webster, 2006). Tilapia do not tolerate as high a dietary lipid level as salmonids. For juvenile *O. aureus*×*O. niloticus*, Chou and Shiau (1996) suggested that a level of 5% dietary lipid appeared to be sufficient to meet the minimum requirement of this tilapia hybrid, but a level of about 12% was needed for maximum growth. However, a dietary lipid level in excess of 12% depressed growth and increased carcass lipid accumulation in juvenile *O. aureus*×*O. niloticus* hybrids (Jauncey and Ross, 1982; Jauncey, 2000). High levels of dietary lipid, especially those rich in polyunsaturated fatty acids, increase the susceptibility of diets to autoxidation and tissue lipid peroxidation, which have been shown to be responsible for detrimental changes of the fatty acid composition of tissues. Tissue accumulation of oxidized breakdown products of lipid can have deleterious consequences for cell and organ functions, as well as depletion of tissue vitamin E concentrations (Stephan et al., 1995; Tocher et al., 2002). The influence of dietary lipid on immune responses and disease resistance of fish has also been demonstrated (Blazer, 1992; Fracalossi and Lovell, 1994; Kiron et al., 1995; Tort et al., 1996; Montero et al., 1998; Balfry and Higgs, 2001; Yildirim-Aksoy et al., 2007).

Tilapia have a dietary requirement for vitamin E (α-tocopherol) and the optimum dietary levels increase with increasing levels of dietary lipid (NRC, 1993; Lim and Webster, 2006). The requirement of *O. aureus* was estimated at 10 mg and 25 mg DL-α-tocopheryl acetate/kg of diet at 3 and 6% dietary lipid, respectively, or 3 to 4 mg *a*-tocopheryl acetate per percent of corn oil (Roem et al., 1990). For *O. niloticus*, the requirement reported was 50 to 100 mg/kg for a diet containing 5% lipid and increased to 500 mg/kg for a diet containing 10 to 15% lipid (Satoh et al., 1987). The dietary vitamin E requirement of tilapia hybrid (*O. niloticus*×*O. aureus*) was 42–44 mg/kg and 60–66 mg/kg in diets containing 5% and 12% lipid, respectively (Shiau and Shiau, 2001). Vitamin E is also known for its role in the prevention of lipid peroxidation

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(McCay and King, 1980; Huang et al., 2003) and functions as a biological antioxidant (Beharka et al., 1997), thus playing an important role in fish immune system function and health. Alpha-tocopherol is particularly abundant in immune cell membranes protecting macrophage membranes from peroxidative damage by free radical produced during respiratory burst (Beharka et al., 1997). Feeding vitamin E depleted diets have been reported to reduce immune responses in several fish species (Blazer and Wolke, 1984; Hardie et al., 1990; Verlhac et al., 1993; Wise et al., 1993; Montero et al., 2001).

Thus, this study was conducted to evaluate the combined effect of increasing dietary lipid levels on vitamin E requirement and their interaction on growth performance, liver α-tocopherol storage, immune responses and resistance of Nile tilapia to *Streptococcus iniae* challenge.

2. Materials and methods

2.1. Experimental fish and rearing facilities

Nile tilapia fry produced at our laboratory that had been reared to juveniles on commercial diets were acclimated to the experimental basal diet for 2 weeks prior to stocking. At the end of the acclimation period, fish (average weight of 7.16 ± 0.18 g) were randomly stocked into 27, 110-L aquaria at 35 fish per aquarium. Aquaria were supplied with flow-through dechlorinated, heated city water at an initial rate of about 0.6 L/min and increased gradually to about 1.0 L/min prior to the end of the study. Water was continuously aerated using air stones. Water temperature and dissolved oxygen in three randomly chosen aquaria were measured once daily in the morning, using a YSI model 58 Oxygen Meter (Yellow Spring Instrument Co., Inc., Yellow Spring, Ohio). During the trial, water temperature averaged 27.9 ± 0.2 °C, and dissolved oxygen averaged 5.1 ± 0.1 mg/L. Photoperiod was maintained at a 12:12 h light:dark schedule.

2.2. Experimental diets and feeding

A basal purified diet was formulated to contain approximately 35% protein and 3.4 kcal DE/g based on the feedstuff values reported in NRC (1993) for channel catfish (Table 1). The basal diet was supplemented with three levels of lipid (combination of equal levels of corn oil and menhaden oil at 6, 10, 14% of diet) and three levels of vitamin E (50, 100, 200 mg/kg diet) as α-tocopheryl acetate (250 mg vitamin E activity/g) at each level of lipid (3×3 factorial experiment). Levels of dextrin and celufil were adjusted to maintain diets isocaloric. Dry ingredients were thoroughly mixed for 10 min in a Hobart mixer before the oil was added. After the oil was diffused into the mix, approximately 250 mL of deionized water/kg of diet was added. The moist mixture was extruded through a 3-mm diameter die in a Hobart meat grinder. The resulting moist pellets were air-dried at room temperature to a moisture content of about 10%. Pellets were ground into small pieces, sieved to obtain approximate sizes and stored frozen in plastic bags at −20 °C until fed. The analyzed levels of lipid and vitamin E of the experimental diets are presented in Table 2.

Fish in three randomly assigned aquaria were fed one of the nine experimental diets twice daily (between 0730–0830 h and 1500–1600 h) to apparent satiation for 12 weeks. During each feeding, feed was offered by hand four to five times until satiation was reached. The amount of diet consumed was recorded daily by calculating the differences in weight of diets prior to the first and after the last feeding. Once a week, aquaria were scrubbed and accumulated waste was siphoned. On cleaning days, fish were fed only in the afternoon. Fish in each aquarium were group-weighted and counted at 3-week intervals. Feed was not offered on sampling days.

2.3. Proximate body composition

At the end of the feeding trial, three fish from each aquarium were randomly collected, euthanized with an overdose of tricaine methanesulfonate (MS-222), pooled and stored at −80 °C for subsequent proximate analysis. After descaling, fish from each aquarium were finely ground in a Hobart meat grinder and analyzed in duplicate for proximate composition following the standard methods (AOAC, 1990). Moisture content was determined by drying samples in an oven at 100 °C until constant weight was reached. Samples used for dry matter were digested with nitric acid and incinerated in a muffle furnace at 600 °C overnight for measurement of ash contents. Protein was measured by combustion method using a FP-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Lipid content of samples was determined by petroleum ether extraction using a Soxtec System (2055 Soxtec Avanti; Foss Tecator, Höganäs, Sweden).

2.4. Hepatosomatic index and liver α-tocopherol content

At the end of the growth trial (12 weeks), livers from four fish per tank that had been bled for serological assays were removed and individually weighed for determination of hepatosomatic index (HSI). In addition to these four livers, livers of three more fish from each tank that had been bled for hematological assays were removed, pooled, and stored at −80 °C for analysis of vitamin α-tocopherol. Vitamin E (α-tocopherol) levels in feeds and livers were analyzed using normal

Table 1
Percentage composition of the basal diet.

Ingredients	Percent in diet
Casein, vitamin-free[a]	32
Gelatin[a]	8
Corn starch[a]	14–40
Carboxymethyl cellulose (CMC)[a]	3
Corn oil + menhaden fish oil (1:1 ratio)[b]	6–14
Vitamin E-free vitamin premix[c]	1
Mineral premix[d]	4
Vitamin E (mg/kg diet)[e]	50–200
Celufil[f]	6–24
Antioxidant (ethoxyquin)	0.02

[a] US Biochemical Corp., Cleveland, OH.
[b] Corn oil was purchased from local grocery store and menhaden fish oil was provided by Omega protein, Inc., Reedville, VA.
[c] The vitamin E-free vitamin mix, diluted in cellulose, provided the following in mg vitamin activity/kg diet: retinyl acetate, 8; cholecalciferol, 2; menadione-sodium bisulfate, 10; thiamin, 10; riboflavin, 20; pyridoxine, 20; D-calcium panthothenate, 200; nicotinic acid, 150; folic acid, 5; cyanocobalamin, 0.02; biotin, 2; choline chloride, 2000; L-ascorbyl-2-polyphosphate (45% vitamin C activity), 222.
[d] Williams and Briggs mineral mix (U.S. Biochemical Corp., Cleveland, Ohio) supplemented in mg/kg diet with aluminum potassium sulfate, 0.7; sodium selenite, 0.08; and cobalt chloride, 1.4.
[e] α-tocopheryl acetate (250 mg vitamin E activity/g).
[f] Non-nutritive filler.

Table 2
Dietary levels of lipid and vitamin E.[a]

Lipid added (%)	Vitamin E added (mg/kg)	Determined levels of	
		Lipid (%)	Vitamin E (mg/kg diet)
6	50	5.35	114.63
	100	5.33	166.83
	200	5.48	254.56
10	50	9.14	110.73
	100	9.05	152.03
	200	9.10	239.62
14	50	12.77	109.14
	100	12.56	154.12
	200	12.86	234.18

[a] Values are means of three and two determinations per diet for lipid and vitamin E, respectively.

phase HPLC with fluorescence detection following the procedures described by BASF Corporation (1997).

2.5. Hematological assays

At the end of the feeding trial, three fish from each tank were collected and anesthetized with tricaine methanesulfonate (MS-222) at 150 mg/L. Blood samples were collected from the caudal vasculature with dried heperinized (100 IU) tuberculin syringes. Hematological assays (red and white blood cell counts, hemoglobin and hematocrit) were performed in duplicate for each sample following the methods described in Lim et al. (2009).

2.6. Immune responses

Serum total protein level, lysozyme activity and natural hemolytic (alternative pathway) complement activity from each of the four fish/aquarium were performed in duplicate as described in Lim et al. (2009). Total serum protein concentration was determined using the modified Biuret method. Serum lysozyme activity was determined by the method of Litwack (1955) as modified by Sankaran and Gurnani (1972) by measuring the lytic activity of the tilapia serum against bacterium *Micrococcus lysodeikticus* (Sigma Chemical Co., St. Louis, MO). Serum alternative complement activity, which is expressed as ACH_{50} (units/mL) and represents the volume of serum necessary to produce lysis of 50% of the target cells under standard conditions, was adapted from Sunyer and Tort (1995) and modified for use in microtiter plates as described in Lim et al. (2009).

2.7. Bacterial challenge and antibody titer

A frozen stock-culture of *S. iniae* (ARS 98-60) from an outbreak of streptococcal disease in Nile tilapia was grown in tryptic soy broth (TSB) at 25 °C with shaking at 100 rpm for 24 h. The concentration of the culture was adjusted to an optical density of 1.0 measured on a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Inc., Norcross, Georgia) at 540 nm to give a *S. iniae* concentration of 1×10^9 colony forming units (CFU)/mL.

At the end of the 12-week feeding period, twenty remaining fish per aquarium were randomly selected and intra-peritoneally (IP) injected with 0.1 mL of 1×10^6 cfu/mL of *S. iniae* (1×10^5 cfu/fish) using a tuberculin syringe. They continued to receive their respective diets. Fish were monitored and mortality was recorded twice daily for 15 days following injection and dead fish were removed.

At the end of the *S. iniae* challenge trial, blood samples were collected from four randomly chosen surviving fish and serum was collected following centrifugation. Agglutinating antibody titers against *S. iniae* in pre- and post-challenge serum samples were determined by modifying the method of Chen and Light (1994) as described in Yildirim-Aksoy et al. (2007).

2.8. Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) using the general linear model (GLM) to test the effects of dietary levels of lipid, vitamin E and their interactions. If there was a significant *F*-test, subsequent comparisons of treatment means were performed using the Duncan's Multiple Range test. Differences were considered significant at the 0.05 probability level. All analyses were performed using SAS statistical software program (SAS Institute, Inc., Cary, NC).

3. Results

Mean final weight gain, dry matter feed intake, feed efficiency ratio (FER) and survival after 12 weeks of feeding with diets containing various levels of lipid and vitamin E are given in Table 3. Weight gain, feed intake and survival were not affected by dietary levels of lipid, vitamin E or their interaction. FER was significantly lower in fish fed 14% lipid diets than in fish fed 6% lipid diets but did not differ from that of fish fed 10% lipid diets. Dietary vitamin E levels or the interaction between vitamin E and lipid levels had no effect on FER.

Table 3
Mean final weight gain, feed intake, feed efficiency ratio (FER, dry matter basis) and survival of Nile tilapia fed purified diets supplemented with various levels of lipid and vitamin E for 12 weeks.[1]

Lipid added (%)	Vitamin E added (mg/kg)	Weight gain (g)	Dry matter feed intake (g/fish)	FER[2]	Survival (%)
6	50	82.29	73.4	1.12	94.3
	100	82.10	72.2	1.14	96.2
	200	75.95	71.4	1.06	98.1
10	50	80.94	74.4	1.09	99.0
	100	80.21	75.3	1.07	99.0
	200	76.12	70.0	1.09	96.2
14	50	72.69	69.3	1.05	95.2
	100	77.37	74.3	1.04	98.1
	200	76.69	73.5	1.04	96.2
Pooled SEM		3.55	2.40	0.02	2.03
Lipid effect (*P* level)		0.2863	0.3916	0.0092	0.4858
6		80.11	73.62	1.11[a]	96.18
10		79.09	71.18	1.08[ab]	98.08
14		75.59	71.28	1.04[b]	96.50
Vitamin E effect (*P* level)		0.4585	0.5321	0.4935	0.6353
	50	78.64	72.41	1.08	96.18
	100	79.89	72.91	1.08	97.77
	200	76.25	70.77	1.06	96.81
Lipid × vitamin E (*P* level)		0.6465	0.3713	0.4201	0.5387

[1]Values are means of three replicates per treatment. Means in the same column with different superscripts are significantly different at $P<0.05$.
[2]FER = Weight gain (g) / dry feed fed (g).

Whole body protein and ash were not influenced by supplemental levels of dietary lipid, vitamin E, or their interaction (Table 4). Whole body moisture and lipid contents significantly decreased and increased, respectively in fish fed 14% lipid diets, but were similar in fish fed 6 or 10% dietary lipid. Increasing the supplemental level of vitamin E to 100 mg/kg diet significantly increased body lipid, but the values of this variable did not differ in fish fed 6 or 14% dietary lipid. Dietary vitamin E content had no effect on body moisture. There was no significant interaction between dietary levels of lipid and vitamin E on body moisture or lipid.

Liver α-tocopherol concentrations significantly increased at each incremental level of dietary vitamin E (Table 4). Increasing dietary lipid levels to 14% significantly reduced liver content of α-tocopherol. The interaction between dietary levels of lipid and vitamin E had no influence on liver content of α-tocopherol. Hepatosomatic index was not affected by dietary levels of lipid, vitamin E or their interaction.

Hematological parameters (red and white blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were not affected by dietary levels of lipid, vitamin E or their interaction (Table 5). Serum protein significantly increased in fish fed 14%-lipid diets but was not affected by supplemental levels of vitamin E (Table 6). Lysozyme activity was not affected by dietary lipid levels but significantly increased in fish fed 200-mg vitamin E diets. Natural hemolytic complement activity significantly decreased in fish fed 10 or 14%-lipid diets but increased when dietary vitamin E levels was increased to 100 or 200 mg. These parameters were not affected by the interaction between dietary levels of lipid and vitamin E (Table 6). Cumulative mortality of fish following *S. iniae* challenge and agglutination antibody titer of fish 15 days post-challenge against that bacterium were not affected by dietary levels of either lipid or vitamin E or their interaction (Table 6).

Table 4
Whole body proximate composition, liver content of α-tocopherol and hepatosomatic index of Nile tilapia fed purified diets containing various levels of lipid and vitamin E for 12 weeks.[1]

Lipid added (%)	Vitamin E added (mg/kg)	Moisture[2] (%)	Percent wet weight basis			Concentration of α-tocopherol[3] (μg/g of liver)	Hepatosomatic index[4] (%)
			Protein[2]	Lipid[2]	Ash[2]		
6	50	71.43	15.92	8.76	3.44	234.02	1.17
	100	70.94	16.06	9.76	2.97	312.70	1.07
	200	70.15	15.87	9.39	3.49	603.00	1.08
10	50	70.32	15.65	9.80	3.17	199.49	1.15
	100	70.17	15.91	10.35	3.21	356.63	1.12
	200	70.42	15.99	9.07	3.12	556.25	1.11
14	50	69.84	15.69	10.50	3.26	135.86	1.14
	100	68.74	15.70	11.24	3.34	274.80	0.99
	200	68.71	15.78	10.68	3.70	496.44	1.11
Pooled SEM		0.67	0.27	0.4	0.18	36.85	0.068
Lipid effect (*P* level)		0.0141	0.5941	0.0006	0.2354	0.0321	0.7442
6		70.84[a]	15.95	9.30[b]	3.30	383.24[a]	1.108
10		70.33[a]	15.84	9.73[b]	3.17	370.79[a]	1.125
14		69.09[b]	15.73	10.81[a]	3.43	302.36[b]	1.082
Vitamin E effect (*P* level)		0.3364	0.7913	0.0476	0.2415	<0.0001	0.2611
	50	70.55	15.76	9.68[b]	3.29	189.79[c]	1.155
	100	69.95	15.89	10.45[a]	3.17	314.71[b]	1.06
	200	69.76	15.88	9.71[b]	3.44	551.89[a]	1.10
Lipid × vitamin E (*P* level)		0.8212	0.9444	0.5548	0.306	0.7456	0.8539

[1]Means in the same column with different superscripts are significantly different at $P<0.05$.
[2]Values are means of two determinations of pooled samples of three fish per tank and three tanks per treatment.
[3]Values are means of livers of five fish per tank and three tanks per treatment.
[4]Values are means of one determination of pooled livers of eight fish per tank and three tanks per treatment. Hepatosomatic index = (liver weight / fish weight) × 100.

4. Discussion

Previous investigations have showed that vitamin E requirements of fish are affected by dietary lipid levels (Watanabe et al., 1977; Lovell et al., 1984; Satoh et al., 1987; Roem et al., 1990; Shiau and Shiau, 2001). Results of the present study shown that, based on weight gain, FI, FER and survival, vitamin E requirement of Nile tilapia was not affected by dietary lipid levels and supplementation of 50 mg of vitamin E/kg diet (analyzed values of 112 mg/kg) was sufficient for tilapia fed purified diets containing an equal mixture of corn oil and menhaden fish oil ranging from 6 to 14%. This value is higher than those determined by Roem et al. (1990) for *O. aureus* (10 and 25 mg/kg of diet at 3 and 6% dietary lipid, respectively, or 3 to 4 mg vitamin E per percent of corn oil) and Shiau and Shiau (2001) for hybrid tilapia (*O. niloticus* × *O. aureus*) (42–44 mg/kg and 60–66 mg/kg in diets containing 5% and 12% lipid, respectively). These values, however, are considerably lower than the requirement values of 50 to 100 mg/kg for a diet containing 5% lipid (pollock liver oil) and 500 mg/kg for a diet containing 10 to 15% lipid reported for Nile tilapia (Satoh et al., 1987). Whether the differences in the degree of fatty acid unsaturation between the lipid source used in this study and that of Satoh et al. (1987) contributed to the differences in the vitamin E requirements cannot be ascertained. However, it has been reported that, in fish, high levels of dietary highly unsaturated fatty acids induced higher vitamin E requirements (Cowey et al., 1983; Roem et al., 1990). It should be noted that our diets were also supplemented with the antioxidant, ethoxyquin, at 200 mg/kg diet.

Table 5
Mean red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of Nile tilapia fed purified diets containing various levels of lipid and vitamin E for 12 weeks.[1]

Lipid added (%)	Vitamin E added (mg/kg)	RBC $\times 10^6$/μL	WBC $\times 10^5$/μL	Hemoglobin[2] (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (%)
6	50	2.20	3.04	9.29	30.93	165.33	50.79	29.93
	100	2.43	3.59	9.58	33.10	136.16	39.51	29.04
	200	2.5	3.39	9.37	32.39	129.42	37.48	28.97
10	50	2.34	2.86	9.25	31.67	135.44	39.50	29.18
	100	2.24	2.88	8.87	31.20	139.11	39.53	28.42
	200	2.19	2.69	9.74	31.00	143.93	45.41	31.51
14	50	2.40	3.39	9.03	30.70	128.47	37.74	29.42
	100	2.13	2.39	8.98	31.10	147.61	42.54	28.89
	200	2.23	2.80	9.39	30.20	135.19	42.07	31.10
Pooled SEM		0.23	0.46	0.40	1.14	17.98	6.27	0.90
Lipid effect (*P* level)		0.7486	0.3251	0.6978	0.3019	0.9037	0.9386	0.7832
6		2.38	3.34	9.41	32.14	143.64	42.59	29.31
10		2.26	2.81	9.29	31.29	139.49	41.48	29.70
14		2.26	2.86	9.13	30.66	137.09	40.78	29.80
Vitamin E effect (*P* level)		0.9667	0.9093	0.5101	0.7209	0.8912	0.9156	0.0863
	50	2.31	3.10	9.19	31.10	143.08	42.68	29.51
	100	2.27	2.95	9.14	31.80	140.96	40.52	28.78
	200	2.31	2.96	9.50	31.19	136.18	41.65	30.52
Fish oil × vitamin E (*P* level)		0.7556	0.5695	0.7557	0.7854	0.6151	0.5145	0.3560

[1]Except for hemoglobin, values are means of two determinations per fish, three fish per tank and three tanks per treatment. Means in the same column with different superscripts are significantly different at $P<0.05$.
[2]Values are means of one determination per fish, three fish per tank and three tanks per treatment.

Table 6
Serum protein, lysozyme and alternative complement activities, cumulative mortality 15 days post-challenge with *S. iniae* and antibody production against the same bacterium of Nile tilapia fed purified diets containing various levels of lipid and vitamin E for 12 weeks.[1]

Lipid added (%)	Vitamin E added (mg/kg)	Serum protein[2] (mg/mL)	Lysozyme activity[2] (μg/mL)	Alternative complement[3] (units/mL)	Cumulative mortality[4] (%)	Antibody titer[2] (log_{10})
6	50	29.24	80.76	71.01	81.5	1.91
	100	29.58	83.55	84.18	83.3	1.98
	200	31.72	98.64	76.74	83.3	1.67
10	50	31.05	89.91	53.32	88.9	2.32
	100	32.13	87.92	56.83	79.6	1.61
	200	31.18	95.57	73.99	87.0	1.31
14	50	32.85	78.93	38.06	88.9	1.94
	100	32.65	91.65	74.77	85.2	2.01
	200	31.27	99.45	70.43	74.1	1.95
Pooled SEM		0.73	4.77	8.04	3.65	0.25
Lipid effect (*P* level)		0.0142	0.6653	0.039	0.6396	0.5766
6		30.17[b]	87.65	77.31[a]	82.7	1.85
10		31.45[ab]	91.13	61.77[b]	85.2	1.75
14		32.26[a]	90.02	61.09[b]	82.7	1.97
Vitamin E effect (*P* level)		0.7951	0.0044	0.0133	0.2551	0.1574
	50	31.05	83.20[b]	54.13[b]	86.4	2.06
	100	31.46	87.71[b]	72.31[a]	82.7	1.86
	200	31.38	97.89[a]	73.72[a]	81.5	1.65
Fish oil × vitamin E (*P* level)		0.1086	0.4305	0.2207	0.0857	0.2831

[1]Means in the same column with different superscripts are significantly different at $P<0.05$.
[2]Values are means of two determinations per fish, four fish per tank and three tanks per treatment.
[3]Values are means of one determination four fish per tank and three tanks per treatment.
[4]Values are means of three tanks per treatment.

Ethoxyquin, although it has no biological activity of vitamin E, can partially spare vitamin E in the diet for growth and other physiological functions (Lovell et al., 1984).

The non-significant effect of increasing dietary lipid levels from 6 to 14% on weight gain, feed intake and survival was expected because our test diets were isonitrogenous and isocaloric and contained essential nutrients at levels that meet or exceed the know requirements for tilapia. A number of earlier studies have reported similar weight gain and FER of channel catfish, *Ictalurus punctatus* (Gatlin and Stickney, 1982; Twibell and Wilson, 2003; Yildirim-Aksoy et al., 2007), blue tilapia (*O. aureus*) (Roem et al., 1990) and European sea bass (*Dicentrarchus labrex*) (Peres and Oliva-Teles, 1999) fed diets supplemented with increasing levels of fish oil. The non-significant effect of dietary fish oil levels on feed intake has also been reported for channel catfish by Twibell and Wilson (2003) and Yildirim-Aksoy et al. (2007). However, significantly lower FER in fish fed diets supplemented with 14% lipid, regardless of dietary levels of vitamin E, may be due to excessive levels of dietary lipid. Lim and Webster (2006) reported that tilapia do not tolerate as high a dietary lipid as do salmonids. A dietary lipid level in excess of 12% depressed growth of juvenile *O. aureus* × *O. niloticus* hybrids (Jauncey and Ross, 1982; Jauncey, 2000). Thus, for good FER, lipid levels in diets of Nile tilapia juveniles should not exceed 10%.

Increasing supplemental lipid levels to 14% significantly increased whole body lipid and decreased moisture, as has been reported for several fish species fed increasing levels of dietary lipid (Jauncey and Ross, 1982; Viola et al., 1988; De Silva et al., 1991; Stowell and Gatlin, 1992; Chou and Shiau, 1996; Peres and Oliva-Teles, 1999; Jauncey, 2000; Yildirim-Aksoy et al., 2007). Other studies, however, showed that carcass lipid of channel catfish (Gatlin and Stickney, 1982) and muscle lipid content of European sea bass (Peres and Oliva-Teles, 1999) and hybrid striped bass (Gaylord and Gatlin, 2000) were not affected by dietary lipid levels. Dietary levels of vitamin E had no effect of body protein, ash and moisture, but body lipid significantly increased in fish fed 100 mg/kg supplemental vitamin E. The significant increase in body lipid of fish fed this diet could not be explained since fish fed diets supplemented with 200 mg supplemental vitamin E had similar body lipid as the group fed 50 mg supplemental vitamin E. Watanabe et al. (1977) and Huang and Huang (2004) reported that whole body proximate composition of common carp (*Cyprinus carpio*) and hybrid tilapia, respectively was unaffected by dietary levels of vitamin E.

Liver concentrations of α-tocopherol significantly increased at each incremental level of dietary vitamin E. Similar findings have been reported for other fish species (Boggio et al., 1985; Hamre et al., 1997; Gatta et al., 2000; Chaiyapechara et al., 2003; Yildirim-Aksoy et al., 2008; Lim et al., in press). However, there was a trend of decreasing liver levels of α-tocopherol with increasing dietary lipid levels, and the value of this variable was significantly lower in fish fed the highest lipid levels (14%). The decrease in tissue α-tocopherol content in fish fed increasing levels of dietary lipid high in $n-3$ HUFA has also been observed in rainbow trout (*Oncorhynchus mykiss*) (Cowey et al., 1984; Boggio et al., 1985), Atlantic salmon (*Salmo salar*) (Hemre and Sandnes, 1999), blue tilapia (Roem et al., 1990), turbot (*Hypsopsetta gutulata*) (Stephan et al., 1995) and Japanese flounder (Wang et al., 2006). It was suggested that vitamin E was increasingly utilized as an antioxidant to protect tissue lipids from oxidation due to greater amounts of $n-3$ HUFA in tissues of fish fed higher levels of dietary lipid.

Earlier studies have shown that Nile tilapia (Satoh et al., 1987), halibut (Tocher, 2003) and gilthead sea bream (*Sparus aurata*) (Mourento et al., 2002) fed diets without vitamin E supplementation had significantly decreased HSI, likely due to tissue degeneration. It has also been found that HSI value is correlated with the amount of fat deposition in livers (Oguri, 1978; Bruslé and Anadon, 1996). Thus, although liver lipid content was not determined in the present study, the non-significant differences among HSI of fish in various treatments may indicate that supplementation of 50 mg vitamin E/kg (112 mg/kg total vitamin E) was sufficient to prevent degeneration and/or fat infiltration of liver tissue in Nile tilapia fed diets containing 6 to 14% lipid. This level of dietary vitamin E was also adequate for normal formation and development of blood cells as none of the hematological parameters evaluated differed among treatments.

Numerous studies have been conducted on the role of dietary lipid sources and essential fatty acids on the immune response and disease resistance in fish, but few studies have dealt with dietary lipid levels and fish health. However, published information on the effect of dietary lipid and essential fatty acids on immune response and disease resistance in fish is inconsistent and often contradictory (Lim et al.,

2008). In the present study, enhanced serum protein concentrations were observed in fish fed 14% lipid diets. This increase in serum protein could be due to elevated lipoprotein levels required for the transport of excess lipid. Serum alternative complement, however, significantly decreased in fish fed 10 or 14% lipid diets. Likewise, Yildirim-Aksoy et al. (2009) obtained significantly increased serum protein and decreased serum complement in channel catfish fed a commercial diet containing 5.6% lipid supplemented with 6% or 9% menhaden fish oil. They also reported increased lysozyme activity in catfish fed diets supplemented with 3 or 6% fish oil. We found, however, that dietary lipid levels had no influence on lysozyme activity of Nile tilapia.

Vitamin E, which is abundant in immune cell membranes (Beharka et al., 1997), plays an important role in the fish immune response (Waagbø, 1994). Peritoneal macrophage function was adversely affected in rainbow trout (Blazer and Wolke, 1984) and channel catfish (Wise et al., 1993) fed vitamin E-deficient diets. Deficiency of vitamin E has been reported to reduce serum protein, serum globulin and phagocyte activity in rainbow trout (Blazer and Wolke, 1984; Clerton et al., 2001) and serum complement activity in Atlantic salmon (Hardie et al., 1990), gilthead seabream (Tort et al., 1996; Montero et al., 1998) and sea bass (Obach et al., 1993). Ortuno et al. (2000, 2001) reported that serum complement and phagocytic activity in gilthead seabream were correlated with dietary levels of vitamin E supplementation, but neither leukocyte migration nor respiratory burst was affected. Lin and Shiau (2005) also obtained significantly higher respiratory burst activity and plasma lysozyme and alternative complement activity in grouper (*Epinephelus malabaricus*) fed diets supplemented with vitamin E ranging from 25 to 800 mg/kg diet than fish fed the unsupplemented control diet. These values progressively increased with increasing levels of dietary vitamin E. In Nile tilapia, Lim et al. (in press) found that supplementation of 50 or 500 mg vitamin E to a basal diet containing 23.1 mg vitamin E/kg had no effect on serum protein, total immunoglobulin and lysozyme activity, but alternative complement was adversely affected at the vitamin E supplemental level of 500 mg/kg. Data of the current study indicated that increasing supplemental levels of vitamin E from 50 mg to 100 or 200 mg/kg diet had no effect on serum protein, but lysozyme and complement activity were stimulated at vitamin E supplemental levels of 200 mg and 100 or 200 mg/kg diet, respectively. Lysozyme activity has been reported to coincide to some degree with a change in the number of the circulating leucocytes (Fletcher and White, 1973; Muona and Soivio, 1992). In the current study, since no difference was found in blood leukocyte count between treatments, the lysozyme production by leukocytes could have been enhanced at high supplemental levels of vitamin E.

Although certain innate immune responses were significantly enhanced by dietary levels of lipid or vitamin E, neither factor nor their interaction affected cumulative mortality of fish 15 days post-challenge with *S. iniae* or antibody production against the same bacterium. A study with channel catfish showed that mortality of fish 14 days post-challenge with *Edwardsiella ictaluri* and antibody titer to *E. ictaluri* did not differ among fish fed a commercial diet (5.6% lipid) with or without supplementation of 3, 6 or 9% menhaden fish oil (Yildirim-Aksoy et al., 2009). With regard to vitamin E, Kim et al. (2003) observed no improvement in the resistance to *E. tarda* challenge of Nile tilapia fed a diet supplemented with 240 mg/kg of vitamin E. Lim et al. (in press) obtained increased resistance to *S. iniae* (lower post-challenge mortality) in Nile tilapia fed diets supplemented with 50 or 500 mg vitamin E/kg (relative to those fed vitamin E-unsupplemented diets containing 23.1 mg vitamin E), but this had no effect on antibody titer. Furones et al. (1992) reported that rainbow trout fed a diet containing 806 mg vitamin E/kg had enhanced resistance but antibody production against *Yersinia ruckeri* was not affected.

Data of the current study show that, based on weight gain, FI, FER and survival, vitamin E requirement of Nile tilapia was not affected by dietary lipid levels and supplementation of 50 mg of vitamin E/kg diet (analyzed average value of 112 mg/kg) was sufficient for Nile tilapia fed diets containing an equal mixture of corn oil and menhaden fish oil ranging from 6 to 14%. However, regardless of the dietary levels of vitamin E, increasing the level of supplemental lipid to 14% resulted in decreased feed efficiency and accumulation of body lipid. Liver content of α-tocopherol increased with increasing dietary level of vitamin E, decreased with increasing dietary levels of lipid and became significantly lower at 14% supplemental lipid. Hematological values and HSI were unaffected by dietary levels of lipid or vitamin E. Dietary lipid levels had no effect on serum lysozyme but an increase in serum protein and a decrease in serum alternative complement activity were observed at 14% and 10 or 14% dietary lipid, respectively. Increasing supplemental levels of vitamin E to 100 or 200 mg/kg diet positively affected serum lysozyme and complement activity but had no influence on serum protein. Even though some immune parameters evaluated were affected by dietary levels of lipid and vitamin E, at levels used, these dietary nutrients appear to be of no benefit in increasing resistance of Nile tilapia to *S. iniae*.

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